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Report on genotyping for identification of reproductive behaviour in Pm21 using marker assisted selection

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Summary

Continuing with the implementation of the guinea-grass breeding scheme (*M. maximus*), a half-sibs population was formed through a factorial cross design. Using standardized protocols, the identification of the reproduction mode was carried out in the population of hybrids obtained (called Pm21) using the molecular marker p779 / p780. A total of 3,156 (44%) hybrids, from 6,820, showed the apomictic marker, exhibiting apomixis: sexual ratio of 1: 1. However, among each of the 198 families formed, the apomixis and sexual ratio varied widely.

Resumen

Continuando con la implementación del esquema de mejoramiento de pasto guinea (*M. maximus*), se conformó una población de medios hermanos mediante un diseño de cruce factorial. Mediante protocolos estandarizados, se realizó la identificación del modo de reproducción en la población de híbridos obtenidos (llamada Pm21) mediante el marcador molecular p779 / p780. De 6,820 híbridos evaluados, 3,156 (44%) mostraron el marcador apomíctico, exhibiendo una relación

apomixis: sexual de 1:1. Sin embargo, entre cada una de las 198 familias formadas, la proporción entre genotipos apomícticos y sexuales vario ampliamente.

Introduction

Continuing with the implementation of guinea-grass breeding program (*Megathyrsus maximus* (Jacq.) B.K. Simon & S.W.L. Jacobs; Syn. *Panicum maximun* Jacq; Simon & S.W.L. Jacobs, 2003), the scheme selected to maximize the genetic gain in a short period of time was the "recurrent selection based on specific combining ability" (RS-SCA). In this scheme it is necessary to identify an appropriate apomictic tester. For this, heterosis and the combining ability must be evaluated in the candidate genotypes. Heterosis describes the phenomenon in which the performance of hybrids, generated from the crossing of two genetically distant genotypes, is significantly higher than the performance of their parents (Birchler et al. 2010). Combining ability is defined as the aptitude of cultivars used as parents to combine with any other genotype, such that desirable genes or traits are passed on to their progeny (Griffing 1956).

During 2019 and 2020 a factorial cross design (24 sexual genotypes x 9 apomictic genotypes) was established to produce a population of hybrids which, when evaluating their performance, will allow measuring the levels of heterosis and combining ability on their parents. To guarantee that the hybrids evaluated are apomictic, it is necessary to identify their reproduction mode. This report summarizes the results obtained when evaluating the reproduction mode in the Pm21 population using the molecular marker p779 / p780, which allows identifying ASGR – BBML gene sequences present in apomictic genotypes in some Paniceae species (Fig. 1I; Worthington et al. 2016).

Materials and Methods

In 2019-2020, a factorial cross between nine apomictic and 24 sexual genotypes was made. Seed was hand-harvested, planted in sand (Fig. 1A) and transplanted individually in plugs after 30 days (Fig. 1B-1D). In 2021, between March and May, leaf tissue from 6,820 hybrids were sampled (Fig. 1F-1G) and the reproduction mode was assessed using the molecular marker p779/p780.

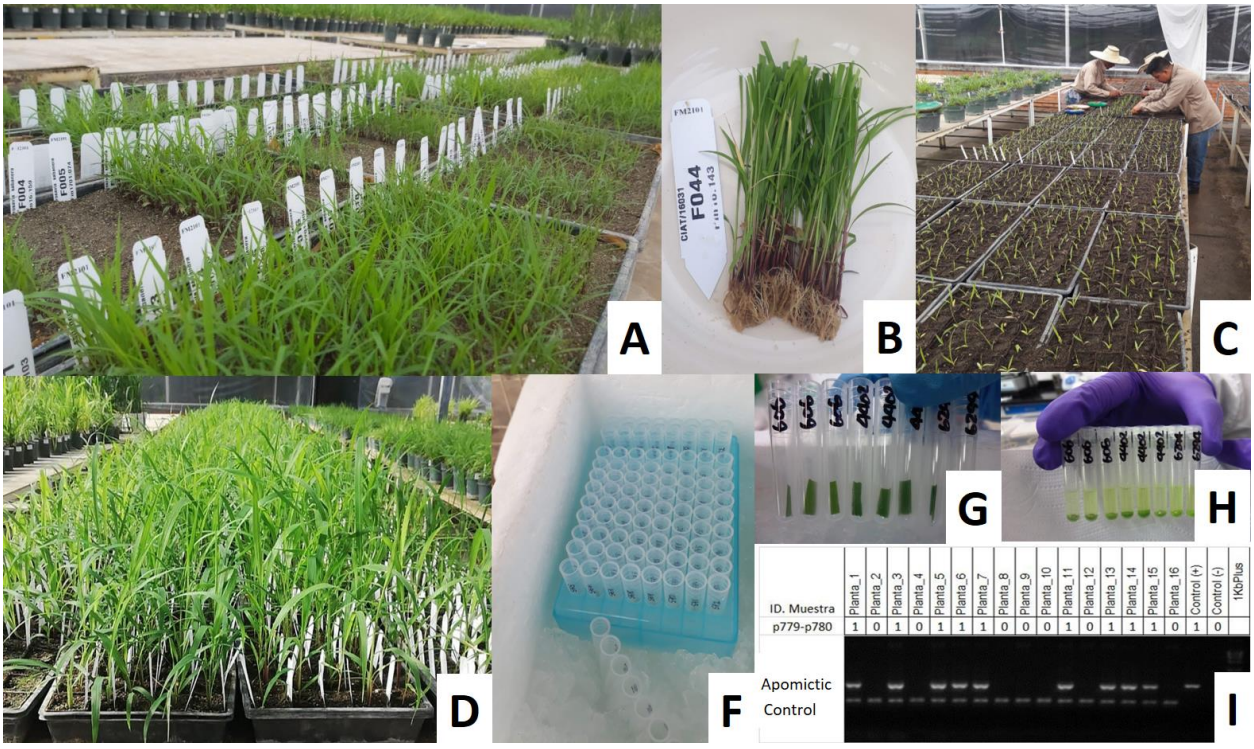

Figure 1. Process to identify apomictic hybrids. A: families planted in sand. B-C: Transplant to plugs. D-H: leaf tissue sample. I: Visualization of molecular marker p779/p780.

Results and Discussion

From 6,820 hybrids, 3,156 (44%) showed the apomictic marker, exhibiting a sexual to apomict ratio of 1:1. Among each of the 198 families formed, the sexual to apomictic ratio varied widely (Table 1). For the following studies, families with high percentage of apomixis (>85%) were discarded based on the premise that they can be apomictic. Very high percentages of apomixis suggest that the genotype used as sexual may be facultative or obligate apomictic. Otherwise, low percentages of apomixis may indicate that there is a low pollination index, a phenomenon that can be influenced by different factors. For example, asynchronous flowering between apomictic and sexual genotypes, low presence of pollinators (*Aphis* sp.) and, scarce wind currents that allows pollen flow.

Table 1. Apomixis percentage expressed in 198 families conformed in a factorial cross among sexual an apomictic genotypes.

Sexual parents	Apomictic parents								
	Sabanera	CIAT/16032	CIAT/26936	CIAT/6799	CIAT/6890	CIAT/6893	CIAT/6986	Mombasa	Naturalizada
Fm1701:002	44.4		14.3	33.3	44.4		26.2		7.1
Fm1701:008		15.6	85.7						
Fm1701:016		38.7	16.7	28.9	62.2	39.5	35.0		23.1
Fm1701:022		5.0	24.4	46.7	31.1			50.0	
Fm1701:025	100.0	61.1	18.2	24.4	55.6	83.3			47.5
Fm1701:033	85.7	80.0	90.9	93.5	65.0	79.5	87.5	97.7	91.7
Fm1701:055		97.8	90.3		95.5	95.6	86.7		66.7
Fm1701:059	48.9	25.0	14.3	40.9	41.5	53.3	47.7	51.4	44.4
Fm1701:074		20.0	20.0	27.6	61.9	40.0	36.4	50.0	39.3
Fm1701:090	45.2	8.7	50.0	51.3	53.5	37.5		16.0	57.1
Fm1701:091		30.0	26.7	17.8	72.7	30.0	40.0		37.2
Pm16:012	44.4	5.0	33.3	32.4	71.9	48.9	34.9	52.4	40.0
PM16:023	44.4	30.6	39.0	28.9	48.9	55.6	54.8	48.0	44.4
Pm16:031	93.3	62.5	95.5	67.6		100.0	37.2		80.0
Pm16:049		16.7	20.0	37.8	37.8		40.9		33.3
Pm16:052				26.7				60.0	
Pm16:055	55.6	26.8	18.6	33.3	27.3	55.6	33.3	44.4	37.1
Pm16:076	48.9	13.3	22.9	39.1	40.0	40.5	37.8		44.0
Pm16:095	37.8		13.3	33.3	53.3		33.3		36.0
Pm16:102		34.1	20.0		43.9	51.1			28.2
Pm16:103			73.3	50.0	59.1	47.7	33.3		35.6
Pm16:104	42.2		35.6	69.2	27.8	18.2	31.1	33.3	
Pm16:111		22.2	35.7	37.5	45.7	42.9	97.8		44.0
Pm16:118	30.0		4.3	40.9	37.5	20.0			
Pm16:124		27.8	36.7	26.5	53.3	73.3	63.3	47.7	44.4
Pm16:130				32.1		93.2	44.4		
Pm16:143	84.4	11.1	17.8	50.0	32.1	51.1	42.2	54.4	47.4
Pm16:150	37.8	9.5	48.9	46.7	37.8	55.6	31.1	42.1	43.2
Pm16:158	31.1		11.1	66.7	37.1		36.6		33.3
Pm16:159				32.3	42.9		57.1		

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